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Rethink carbohydrase: Answering single vs. multi-enzyme question

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THE use of carbohydrase enzymes in monogastric diets has increased dramatically in recent years, achieving market penetration of close to 100%.

Carbohydrase use in poultry and pig diets is driven by two main factors: (1) the effect of such enzymes on the energy utilization of the diet, allowing for a reduction in the energy content (and cost) while maintaining animal performance, and (2) the impact on lower gut fermentation, leading to better gut health and improved performance.

As discussed in a previous article (*Feedstuffs*, Sept. 2), the main hypothesis regarding how these effects are achieved is through the provision of prebiotics and reductions in the cage effect and viscosity in the gut.

The range of products available in the market can be classified as either "single" enzymes, with just one target activity, or more "complex" multi-enzymes, which can either be a fermentation product with declared multiple activities or a blend of two or more fermentation products with main activities and several non-target activities. The comparison between single- and multi-enzyme products is routinely discussed in both scientific and commercial literature.

The main argument for the use of a multi-enzyme product comes from the hypothesis that, since the fiber composition present in a diet is variable and consists of several different structures, several enzymes would be necessary to disrupt this fiber, increase digestibility and open up cell walls to reduce the cage effect, allowing the entrapped nutrients inside to be digested. As discussed in the previous article, this approach lacks scientific support, because the cage effect is potentially a secondary outcome of the retention time of the digesta in the gut rather than a direct result of the enzyme activity.

Furthermore, if multi-enzymes were to extract the energy value in fiber by increasing its digestibility at the terminal ileum, then very little of this would be through fermentation, given that the vast majority of the fermentative capacity is in the large intestine. Indeed, observations of the effects of enzymes for non-structural polysaccharides (NSPs) have concluded that the benefits in fiber digestion occur in the large intestine, which suggests that digestion of cell walls *per se* does not take place early on in the small intestine — again questioning the cell wall hypothesis that relies on the early release of entrapped nutrients.

Single- and multi-enzyme products are a semantic classification, implying that "single" enzymes are a purified product that have only one activity, while "multi" enzymes would have one or more target/controlled activity and additional activities within the product that could bring extra value to the final product. The reality, though, is not necessarily so simple, as single-enzyme products also present side enzyme activities that are considered irrelevant to the activity of the product and, thus, are not reported or controlled during production.

Regardless, these side activities are usually less stable through storage, and processing (especially pelleting) would further reduce their activity, again, to irrelevant levels. As an example, O'Neill (2014) reported that the side activity of glucanase and cellulase in Econase XT (an enzyme classified as a single xylanase) was reduced to 3% and 5%, respectively, of initial levels after pelleting. On the other hand, very high levels of xylanase activity have been observed in a product claiming to be a "single" mannanase product.

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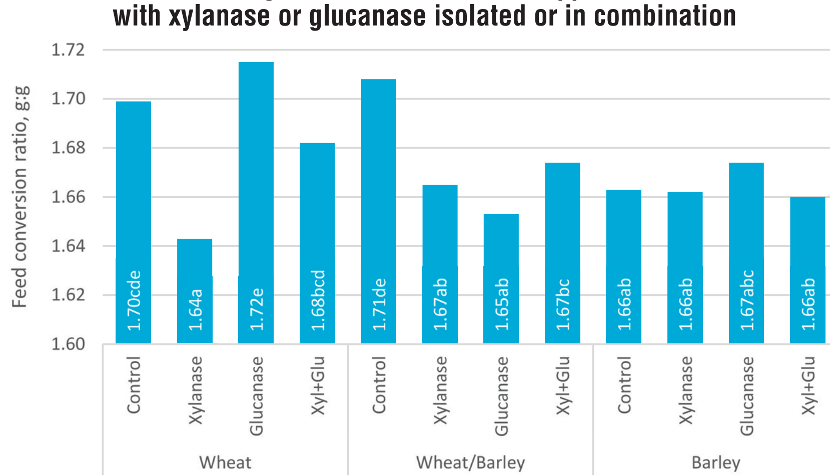
Hypothetical experimental treatment design to test three-enzyme combination product

Treatment	Enzyme
Control	No enzyme
1	Enzyme 1
2	Enzyme 2
3	Enzyme 3
4	Enzyme 1 + Enzyme 2
5	Enzyme 2 + Enzyme 3
6	Enzyme 1 + Enzyme 2 + Enzyme 3

Note: The number of treatments needed (including the control) for testing a five-enzyme combination product, for example, would be 121.

Feed conversion ratio (g:g) of 42 day-old broilers fed diets containing different cereals and supplemented with xylanase or glucanase isolated or in combination



a-e Means followed by different superscript letters are different by least-significant difference test ($P < 0.05$).

Source: Adapted from Ten Doeschate, 2013.

Whenever a multi-enzyme is reported, though, the activity of these other activities (and sometimes even some of the main activities) in the final feed are very rarely reported, so it is not even possible to determine if all of the multi-enzyme activities are actually present in the diet fed to animals.

In a meta-analysis by Torres Pitarch et al. (2019), 560 publications were reviewed to determine the effect of single- and multi-enzyme products on pig performance. From this database, 67 publications were used in the final analysis (a 12% usage rate that already suggests significant problems in running such an analysis), but the enzyme activity in the feeds was not reported as one of the parameters in the evaluation. This questions the validity of a comparison between products claiming to be single- versus multi-enzyme.

The result of the meta-analysis showed that single xylanase products improved average daily gain when fed in diets based on corn and co-products, reduced intake when fed in diets based on rye and tended to reduce intake when fed in diets based on wheat. On the other hand, when xylanase-plus-glucanase products were evaluated, no effect whatsoever was observed on any diet. When a complex of enzymes was tested, performance was improved in diets rich in corn, wheat, barley and co-products, but there was no effect in rye and sorghum-based diets.

Biologically, these results make no sense, as they suggest that there was sometimes an effect when a xylanase product was fed, but this effect was lost by the inclusion of glucanase yet reappeared when an even more complex enzyme product (usually containing xylanase and glucanase) was used.

O'Neil (2014) and Bedford (2018) addressed the difficulty of evaluating such products, suggesting that all enzyme activities declared should be analyzed in both the product and the final feed offered to the animals (covering any thermostability and dilution factor). An activity would only be credited to the enzyme product if it was found at a level greater than that of the endogenous activity from the cereals used to produce the feed. The effect of inclusion of more than one enzyme activity would have to be determined by adding each enzyme in isolation (at the same level and origin of the enzyme used in the multi-enzyme product) and adding them together to observe if there was any additional benefit in the animal when the enzymes were combined compared with each in isolation (Table).

Whenever such evaluations have been conducted in broilers (Cowieson, 2010; Ten Doeschate, 2013, as shown in the Figure; Walk, 2013; Dos Santos, 2015; Stefanello, 2015; Amerah, 2017) and pigs (Torres Pitarch, 2019), the inclusion of several enzyme activities has not demonstrated a consistent beneficial effect over and above that of an effective single-enzyme product (routinely xylanase), which questions the need for several enzyme activities to optimize animal performance.

As an evolution of the original viscosity/prebiotic/cage effect hypothesis, it has been suggested that viscosity reduction, coupled with increasing fiber solubility and the production of low-molecular weight oligosaccharides, allows the microbiota in the lower gut to further develop its capacity to ferment fiber. Development of this fiber-fermenting microbiome would increase the production of volatile fatty acids such as butyrate, propionate and acetate that have a beneficial effect on intestinal structure and digesta transit time and so improve nutrient absorption and animal performance.

A better understanding of the effect of a carbohydrase in animal nutrition, thus, requires knowledge of not just the enzyme but the quality of the ingredients and the fiber composition of the diet, since it is the combination that dictates the production of fermentable, soluble fiber, which influences both the development of the gut microbiome and overall gut health.

This conclusion supports the statement by Choct (2015): "In the future, new nutrient matrices must be developed to account for all carbohydrates in the ingredients, including amount and types of oligosaccharides and NSP (soluble, insoluble and total NSP). Such an approach will lead to a more targeted use of nutraceuticals such as NSP-degrading enzymes, fiber additives and other gut enhancers."

References

The list of references may be obtained by emailing nam@abvista.com. ■